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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/444,335	11/19/99	ENIKOLOPOV G	CSHL99-05

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EXAMINER	
SCHNIZER,R	
ART UNIT	PAPER NUMBER
1632	17

DATE MAILED: 06/20/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.	Applicant(s)	
	09/444,335	ENIKOLOPOV ET AL.	
	Examiner	Art Unit	
	Richard Schnizer	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 April 2001* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-79* is/are pending in the application.

4a) Of the above claim(s) *25-50* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-24 and 51-79* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15)☒ Notice of References Cited (PTO-892)
16)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17)☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
18)☐ Interview Summary (PTO-413) Paper No(s). _____ .
19)☐ Notice of Informal Patent Application (PTO-152)
20)☐ Other: .

DETAILED ACTION

An amendment and an information disclosure statement were received and entered as Paper Nos 15 and 16 on 4/9/01, and 5/16/01, respectively. Claims 51-79 were added as requested. Claims 1-79 are pending. Claims 25-50 were withdrawn from consideration in Paper No. 13, as being drawn to a non-elected invention. Claims 1-24 and 51-79 are under consideration in this Office Action.

Objections Withdrawn

The objections to claims 17, 22, 23, and 24 are withdrawn in view of Applicant's amendments and argument.

Rejections Withdrawn

After further consideration, the rejection of claims 1-17, 19-24 under 35 U.S.C. 112, first paragraph is withdrawn.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-17, 19-24, and 51-79 are rejected under 35 U.S.C. 102(b) as being anticipated by Zimmerman et al (1994), as evidenced by Hogan (1986) for the reasons of record in Paper No. 13.

Response to Arguments

Applicant's arguments filed 4/9/01 have been fully considered but they are not persuasive.

The issue is whether or not beta galactosidase is a "marker fluorescent protein". The specification provides no limiting definition for the term "marker fluorescent protein", but discloses that "a marker/reporter protein (e.g. a fluorescent protein)" may be used in the claimed invention. See, for example, page 3, lines 11-16. The Examiner has established that beta galactosidase is a fluorescent protein based on the fact that it comprises numerous tryptophan residues. In response, Applicant argues that fluorescent proteins have excitation and emissions spectra which differ from the fluorescence spectra of the tryptophan residues, "[t]hus the fluorescence (excitation/emission spectral features) of "fluorescent proteins" are [sic] distinguishable from the fluorescence of tryptophan in a protein." This argument is not persuasive because it fails to establish that beta galactosidase is not a marker fluorescent protein. Applicant's arguments noting documented differences between beta galactosidase and green fluorescent protein also fail to establish that beta galactosidase is not a marker fluorescent protein. For these reasons the rejection is maintained.

With respect to new claims 72-77, Applicant argues that Zimmerman does not teach a method for measuring cells in a live or adult animal or an organ thereof. In response, it is noted that claims 72-77 do not require that the animal must be alive during the measurement steps, nor that the fluorescence of the fluorescent protein must be measured. Zimmerman clearly teaches the detection of a population of neuronal stem cells in an animal. These cells clearly fluoresce because they contain fluorescent materials such as proteins. Absent evidence to the contrary, this population of cells was present in the animal when it was alive, prior to the measurement step. Thus, Zimmerman teaches a method of indirectly measuring a population of neuronal stem cells in a live animal. The rejection of claims 72-77 under 35 USC 102 can be overcome by amending the claim to require that the measurement steps must be carried out on a live animal, or in a live organ or tissue thereof, and that the fluorescence of the fluorescent protein must be measured. Applicant's argument that Zimmerman does not teach a method of measuring multipotent stem and progenitor cells in an adult animal is irrelevant because no such method is claimed.

Claim 79 is directed to an adult transgenic mammal which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding a fluorescent protein, wherein the fluorescent protein is expressed in multipotent stem and progenitor cells of the adult animal. These characteristics are considered to be inherent in the adult mice of Zimmerman which were used to maintain the transgenic lines. See page 14, column 1, lines 3-7.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-24, 51-71, 78, and 79 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zimmerman (1994) in view of Chiochetti (1997) for the reasons of record in Paper No. 13.

Response to Arguments

Applicant's arguments filed 4/9/01 have been fully considered but they are not persuasive.

Applicant appears to question the motivation to combine the cited references, and implies that any such motivation was taken from the instant disclosure rather than the prior art. Applicant's attention is directed to the abstract, and page 202, column 1, lines 5-7 of Chiochetti, which teach that green fluorescent protein is a more powerful and sensitive tool for studying gene expression in transgenic animals than is beta galactosidase. Applicant has failed to make clear why this would not provide motivation to one of ordinary skill in the art to substitute a sequence encoding green fluorescent protein for the sequence encoding beta galactosidase in the construct and methods of Zimmerman.

Applicant also argues that the teaching of Zimmerman is not directed to the problem of studying or measuring multipotent stem and progenitor cells, nor in producing a non-human

transgenic animal that would allow studying such cells or measuring their presence. This argument flies in the face of the disclosure of Zimmerman who teaches a method of making a transgenic animal comprising a lac-Z transgene under control of the promoter and second intron enhancer of the rat nestin gene, and the detection of neuronal stem cells in these animals. See entire document, especially abstract; Table 1, pages 12 and 13, particularly constructs B, C, and F; Fig. 2 on page 15; and page 23, fourth full paragraph. Applicant has provided no reason why one of ordinary skill in the art, in view of the Chiochetti's disclosure that green fluorescent protein is superior to beta galactosidase, would not substitute green fluorescent protein in the the construct and methods of Zimmerman.

Claims 51-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zimmerman (1994) in view of Chiochetti (1997), Yeh et al (Proc. Nat. Acad. Sci. USA 92:7306-7040, 7/1995), Lois et al (Science 264(5162):1145-1148, 5/1994), and Reynolds et al (Science 255(5052):1707-1710, 3/1992).

Zimmerman who teaches a method of making a transgenic animal comprising a lac-Z transgene under control of the promoter and second intron enhancer of the rat nestin gene, and the detection of neuronal stem cells in these animals for the purpose of analyzing gene expression in these cells. See entire document, especially abstract; page 11, last sentence of paragraph bridging columns 1 and 2; sentence bridging pages 11 and 12; Table 1, pages 12 and 13, particularly constructs B, C, and F; Fig. 2 on page 15; and page 23, fourth full paragraph.

Zimmerman does not teach a method of measuring multipotent stem and progenitor cells wherein the measurement step is carried out in a live animal.

Chiochetti teaches that green fluorescent protein (GFP) is a more powerful and sensitive tool for studying gene expression in transgenic animals than is beta galactosidase. See entire document, especially page 202, column 1, lines 5-7. Chiochetti also teaches that GFP allows direct imaging in living cells, and suggests that changes in gene expression in living tissues could be examined. See page 201, column 1, lines 11-14.

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Zimmerman by substituting green fluorescent protein for beta galactosidase, and to study gene expression in neuronal stem cells in living animals and their organs and tissues. One would have been motivated to do so because Chiochetti teaches that GFP allows direct imaging in living cells, and suggests that changes in gene expression in living tissues could be examined through the use of GFP. See page 201, column 1, lines 11-14. Furthermore, Yeh teaches that GFP can be monitored in intact, living embryos, and can be used for a variety of purposes including measurement of dynamic changes in gene expression in living tissue; lineage analysis; and monitoring cell migrations and changes in cell shape. See abstract; the last two sentences of the first full paragraph of column 2 on page 7036; page 7040, column 1, lines 5-9, and first two sentences of paragraph bridging columns 1 and 2 on page 7040. In addition, Lois et al teach the study of migration of neuronal precursors in adult mammalian brain, and Reynolds teaches that adult neuronal stem cells express nestin. See abstracts. Given the

teachings of the prior art as discussed above, one of ordinary skill in the art would have been motivated to use a transgenic animal comprising a GFP sequence under the control of nestin regulatory sequences to follow neuronal precursor migration in living animals. In summary, the prior art provides explicit statements that GFP is superior to beta galactosidase for use in transgenic animals, and that it should be used to study cells of transgenic animals *in vivo*. The prior art also teaches that nestin transcription control sequences can be used to study neuronal precursor cells, and provides motivation to examine the transcriptional activities of these cells as well as their migration and morphology. Armed with this information, one of ordinary skill in the art would clearly be motivated to use nestin/GFP constructs in transgenic animals for the purpose of studying neuronal precursor populations.

Thus the invention as a whole was *prima facie* obvious.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after

the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 103-306-5441. The examiner can normally be reached Monday through Friday between the hours of 6:20 AM and 3:50 PM. The examiner is off on alternate Fridays, but is usually in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Karen Hauda, can be reached at 703-305-6608. The FAX numbers for art unit 1632 are 703-308-4242, and 703-305-3014.

Inquiries of a general nature or relating to the status of the application should be directed to the Patent Analyst Patsy Zimmerman whose telephone number is 703-308-8338.

Scott D. Priebe

Richard Schnizer, Ph.D.